



Faculty of Science
Biochemistry Department

Evaluation of the Therapeutic Potential of Bone Marrow Derived- Mesenchymal Stem Cells in an Experimental Model of Lung Fibrosis

A thesis submitted for the degree of Ph.D. in Biochemistry

Submitted by

Alyaa Saher Abd El Halim

(M.Sc. in Biochemistry 2015)

Assistant Lecturer of Biochemistry - Faculty of Science - Ain Shams University

Under the supervision of

Prof. Dr. Mohamed R. Mohamed

Professor of Biochemistry & Molecular Biology
Faculty of Science
Ain Shams University

Prof. Dr. Hanaa H. Ahmed

Professor of Biochemistry
Hormones Department
Medical Research Division
National Research Centre

Dr. Hadeer A. Aglan

Researcher of Biochemistry
Hormones Department
Medical Research Division
National Research Centre

**Faculty of Science
Ain Shams University
2020**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

سَبِّحْكَ لَا إِلَهَ إِلَّا
أَنْتَ عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

صدق الله العظيم

سورة البقرة الآية: ٣٢

This thesis has not been submitted
before to this or any other University

Alyaa Saher Abd El Halim

ACKNOWLEDGEMENT

No words can express my sincere gratitude to *Prof. Dr. Mohamed Ragaa Mohamed*, for his meticulous supervision, sincere guidance, constructive suggestions and wholehearted moral support throughout this work.

Special thanks are extended to *Prof. Dr. Hanaa Hamdy Ahmed*, for giving me the privilege of working under her valuable supervision, for her constant support and fruitful comments at every stage of this work.

I owe my sincere thanks and gratitude to *Dr. Hadeer Ahmed Ahmed Aglan*, for her kind supervision, great support, instructive guidance, valuable technical assistance and helpful advice.

I also express my sincere appreciation to *Prof. Dr. Adel Bakeer Kholoussy*, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University, for his kind cooperation in conducting histological examination.

Alyaa Saher Abd El Halim

Contents

Contents	Page
➤ List of abbreviations	i
➤ List of figures	iv
➤ List of tables	viii
➤ Abstract	ix
➤ Introduction	1
➤ Aim of the work	7
➤ Review of literature	8
Pulmonary fibrosis	8
Epidemiology of pulmonary fibrosis	8
Risk factors for pulmonary fibrosis	10
Clinical manifestations and natural history of pulmonary fibrosis	11
Diagnosis of pulmonary fibrosis	12
1. Clinical evaluation	12
2. History	13
3. Laboratory testing	13
4. Chest imaging	14
5. Tissue evaluation	14
Pathogenesis of pulmonary fibrosis	14
1. Proliferation of resident fibroblasts	17
2. Epithelial-mesenchymal transition	17
3. Bone marrow-derived fibrocytes	18
Inflammatory response during the development of pulmonary fibrosis	22
Extracellular matrix remodeling in pulmonary	

fibrosis	22
Alveolar epithelium damage in pulmonary fibrosis	
pathogenesis	23
Pulmonary epithelial repair	24
TGF- β 1/Smad3/S100A4 signaling in pulmonary	
fibrosis	25
Treatment of pulmonary fibrosis	27
I. Pharmacologic therapies	27
1) Pirfenidone	28
2) Nintedanib	28
II. Cell-based therapies	29
Types of cells used in cell-based therapies of	
pulmonary fibrosis	30
1. Alveolar epithelial cells	30
2. Lung mixed epithelial cells	31
3. Disease-specific human-induced pluripotent	
stem cells	31
4. Endogenous lung stem cells	32
5. Circulating endothelial progenitor cells	33
6. Stem cells	33
Mesenchymal stem cells	39
1. Bone marrow-derived mesenchymal stem	
cells from	42
2. Adipose tissue-derived mesenchymal stem	
cells	42
3. Embryonic cord- and placenta-derived	
mesenchymal stem cells	43
Mesenchymal stem cells for the treatment of	
pulmonary fibrosis	45
Mechanisms of action of mesenchymal stem cells	48
1. Homing	50
2. Differentiation	50
3. MSCs secretome	51
a) Soluble factors	56
• Growth factors	56
• Anti-inflammatory cytokines	59

b) Extracellular vehicles	62
Conditioned media	66
MSC-derived secretome pharmaceuticalization	67
Integration of stem cell-based treatments into the clinical management of pulmonary fibrosis	67
Challenges towards clinical use of mesenchymal stem cells in the treatment of inflammatory lung diseases	70
➤ Materials and methods	73
Isolation, purification and characterization of rat BM-MSCs	73
Labelling of rat BM-MSCs	75
Preparation of mesenchymal stem cells conditioned medium	76
Amiodarone-induced pulmonary fibrosis model	76
Experimental animal groups and treatments	79
<i>Collection of blood samples and lung tissues</i>	81
Histopathological procedure	81
Measurement of serum MIP2 levels	82
Measurement of serum CC16 levels	86
Measurement of serum KGF levels	89
Quantitative analysis of genes expression	93
I. RNA extraction	93
II. cDNA synthesis	98
III. Gene expression assay	100
Statistical analysis	104
➤ Results	105
Morphological and immunophenotypic characterization of the BM-MSCs	105
Evaluation of lung histological alterations	108
Assessment of collagen fibers	117

Tracking of ferumoxides-labeled BM-MSCs	126
Serum concentrations of MIP2	130
Serum concentrations of CC16	132
Serum concentrations of KGF	135
Gene expression analysis of COL1A1 and CTGF	138
Gene expression levels of TGF- β 1, SMAD3 and S100A4	142
➤ Discussion	147
➤ Summary	157
➤ References	161
➤ الملخص العربي	
➤ المستخلص	

List of abbreviations

Abbreviations	Full name
ABM	Alveolar basement membrane
ACTB	β -actin
AD	Amiodarone
AEC1	Type 1 alveolar epithelial cells
AEC2	Type 2 alveolar epithelial cells
AECs	Alveolar epithelial cells
AMSCs	Adipose tissue-derived mesenchymal stem cells
Ang1	Angiopoietin 1
ANOVA	One-way analysis of variance
ASCs	Adult stem cells
BAL	Bronchoalveolar lavage
BLM	Bleomycin
BM-MSCs	Bone marrow-derived mesenchymal stem cells
CC16	Clara cell secretory protein
CCL	Chemokine (C-C motif) ligand
CCR	C-C chemokine receptor type
CD	Cluster of differentiation
cDNA	Complementary DNA
CM	Conditioned media
COL1A1	Type I collagen
COPD	Chronic obstructive pulmonary disease
CTGF	Connective tissue growth factor
CXCL	C-X-C motif chemokine ligand
CXCR	C-X-C chemokine receptor
DL _{CO}	Diffusing capacity of lungs for carbon monoxide
DMEM	Dulbecco's Modified Eagle's medium
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay

EMT	Epithelial to mesenchymal transition
EP2	Prostaglandin E2 receptor 2
EP4	Prostaglandin E2 receptor 4
EPCs	Endothelial progenitor cells
ER	Endoplasmic reticulum
ESCs	Embryonic stem cells
EVs	Extracellular vesicles
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate
FVC	Forced vital capacity
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte/macrophage colony stimulating factor
GSK	Glycogen synthase kinase
H&E	Hematoxylin and eosin
HAECs	Human amniotic epithelial cells
HGF	Hepatocyte growth factor
HO-1	Hemeoxygenase 1
HRCT	High-resolution computed tomography
HRP	Horseradish peroxidase
HSC	Hematopoietic stem cell
IDO	Indoleamine 2,3-dioxygenase
IGF-1	Insulin like growth factor 1
IL	Interleukin
IL-1Ra	Interleukin 1 receptor antagonist
IPF	Idiopathic pulmonary fibrosis
iPSCs	Induced pluripotent stem cells
IQR	Interquartile range
JNK	c-Jun N-terminal kinase
KGF	Keratinocyte growth factor
LIF	Leukemia inhibitory factor
L-MSCs	Lung mesenchymal stem cells
MCP-1	Monocyte chemoattractant protein 1
MIP-2	Macrophage inflammatory protein 2
MMP	Matrix metalloproteinase

MPCs	Mesenchymal progenitor cells
MSCs	Mesenchymal stem cells
NF- κ B	Nuclear factor kappa light chain enhancer of activated B cells
NK	Natural killer cells
OD	Optical density
PAH	Pulmonary arterial hypertension
PBS	Phosphate-buffered saline
PDGF	Platelet derived growth factor
PF	Pulmonary fibrosis
PGE2	Prostaglandin E2
PKC	Protein kinase C
PLL	Poly-L-lysine
PS	Phosphatidylserine
qPCR	Quantitative real-time polymerase chain reaction
RT	Reverse transcription
S100A4	S100 calcium-binding protein A4
SD	Standard deviation
SDF-1	Stem cell derived factor-1
SMAD	Mothers against decapentaplegic homolog
STC-1	Stanniocalcin 1
TGF- β 1	Transforming growth factor beta 1
TIMP	Tissue inhibitor of metalloproteinase
TNF- α	Tumor necrosis factor- α
TSG-6	Tumor necrosis factor-stimulated gene 6
uMSCs	Umbilical cord mesenchymal stem cells
VEGF	Vascular endothelial growth factor
α -SMA	Alpha-smooth muscle actin

List of figures

Figure No.	Legend	Page
1	The events underlying the pathogenesis of pulmonary fibrosis	21
2	Stem cell division in relation to self-renewal and the repopulation potential	35
3	The main sources of stem cells used for the development of cellular therapies for pulmonary fibrosis	37
4	Homing of intravenously- or intratracheally-delivered mesenchymal stem cells to the sites of injury in the lungs	38
5	Properties of mesenchymal stem cells	40
6	Potential sources of MSCs in lung repair	41
7	Delivery and mode of action of intravenously- delivered MSCs into the injured lungs	49
8	Differentiations of MSCs after homing to the injured lung	52
9	Multiple therapeutic effects of MSCs secretome involved in lung regeneration	55
10	Anti-inflammatory cytokines secreted by MSCs	61
11	Extracellular vesicles released by MSCs	63
12	Extracellular vesicles secreted by MSCs transfer their cargo to the recipient cells	64
13	The integration of stem cell-based treatments into the clinical management strategy of PF	69
14	Structure of amiodarone	78
15	Passage three BM-MSCs appeared as spindle-shaped fibroblast-like flattened cells	106

16	Immunophenotypic analysis of BM-MSCs	107
17	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from vehicle (saline)-administered rats	109
18	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from AD-administered rats	110
19	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 1 month post-BM-MSCs-treated rats	111
20	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 2 months post-BM-MSCs-treated rats	112
21	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 4 months post-BM-MSCs-treated rats	113
22	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 1 month post-CM-treated rats	114
23	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 2 months post-CM-treated rats	115
24	A representative optical micrograph (×100) of hematoxylin & eosin-stained lung sections from 4 months post-CM-treated rats	116
25	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from vehicle (saline)-administered rats	118

26	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from AD-administered rats	119
27	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 1 month post-BM-MSCs-treated rats	120
28	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 2 months post-BM-MSCs-treated rats	121
29	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 4 months post-BM-MSCs-treated rats	122
30	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 1 month post-CM-treated rats	123
31	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 2 months post-CM-treated rats	124
32	A representative optical microphotograph (×100) of Masson's trichrome-stained lung sections from 4 months post-CM-treated rats	125
33	A representative optical microphotograph of Prussian blue-stained lung sections from 1-month post-BM-MSCs-treated rats	127
34	A representative optical microphotograph of Prussian blue-stained lung sections from 2-month post-BM-MSCs-treated rats	128
35	A representative optical microphotograph of Prussian blue-stained lung sections from 4-month post-BM-MSCs-treated rats	129

36	Serum concentrations of MIP-2 in vehicle- and AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	131
37	Serum concentrations of CC16 in saline- and AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	134
38	Serum concentrations of KGF in saline- and AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	137
39	Pulmonary tissue mRNA levels of COL1A1 in AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	140
40	Pulmonary tissue mRNA levels of CTGF in AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	141
41	Lung tissue mRNA levels of TGF- β 1 in AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	144
42	Lung tissue mRNA levels of SMAD3 in AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	145
43	Lung tissue mRNA levels of S100A4 in AD-administered rats as well as at 1, 2 and 4 months post-BM-MSCs and -CM treatments	146